Glucose Uptake Assay Kit

Catalog No.: abx295021

Size: 96 tests

Detection Range: 0.02 nmol/µl – 0.3 nmol/µl

Sensitivity: 0.02 nmol/µl

Storage: Store all reagents at -20°C. Store the Chromogenic Reagent in the dark.

Application: For detection and quantification of Glucose Uptake in living cells.

Introduction

Glucose is the most abundant monosaccharide, and is central to the metabolism of organisms in all known clades. The rate of glucose uptake is a key indicator of cellular health and behavior, and offers insights into cellular processes in a vast array of research areas. Glucose uptake be affected by cellular stressors such as disease, inflammation, the presence of metabolic toxins, and environmental conditions. As such, glucose uptake is of interest to researchers studying both micro- and macrobiological processes.

Abbexa's Glucose Uptake Assay Kit is a quick, convenient, and sensitive method for measuring and calculating glucose uptake in living cells. When using this kit, the glucose chemical analogue 2-deoxyglucose (2-DG) is provided to the cells, which is taken up by glucose transporters and metabolized into 2-deoxyglucose-6-phopshate (2-DG-6P). The process of metabolizing 2-DG reduces NADP+ to NADPH. NADPH acts as cofactor to allow the enzyme diaphorase to produce a fluorescent compound, with an excitation wavelength of 530 nm (emission wavelength 590 nm). The intensity of the fluorescence is proportional to the amount of glucose taken up, which can then be calculated.

Kit components

1. 96-well black (fluorescence) microplate
2. Acidic Reagent: 10 ml
3. Alkaline Reagent: 10 ml
4. Chromogenic Reagent: 25 ml
5. Enzyme Reagent: 2 vials
6. Substrate: 1.5 ml
7. Standard (0.3 nmol/µl): 2 ml
8. Buffer Solution: 55 ml
9. Plate sealer: 2

Materials required but not provided

1. Fluorescence plate reader (excitation wavelength 530 nm, excitation wavelength 590 nm)
2. Blank 96-well microplate
3. Distilled water
4. Bovine serum albumin (BSA) powder
5. Pipette and pipette tips
6. Vortex mixer
7. Incubator

Protocol

A. Preparation of reagents

1. Reagents

- **Enzyme Reagent Working Solution:** Dissolve a vial of powdered Enzyme Reagent with 10 ml of Chromogenic Reagent. Mix fully. The Enzyme Reagent Working Solution can be stored for up to 3 days at 4°C in the dark.
- **Standards:** Label 7 tubes with 0.3 nmol/µl, 0.24 nmol/µl, 0.21 nmol/µl, 0.18 nmol/µl, 0.15 nmol/µl, 0.12 nmol/µl, and 0.06 nmol/µl. Add 200 µl, 160 µl, 140 µl, 120 µl, 100 µl, 80 µl, and 40 µl of Standard (0.3 nmol/µl) to the 0.3 nmol/µl, 0.24 nmol/µl, 0.21 nmol/µl, 0.18 nmol/µl, 0.15 nmol/µl, 0.12 nmol/µl, and 0.06 nmol/µl tubes respectively, followed by 0 µl, 40 µl, 60 µl, 80 µl, 100 µl, 120 µl, and 160 µl of Distilled water, to prepare Standard Dilutions with concentrations 0.3 nmol/µl, 0.24 nmol/µl, 0.21 nmol/µl, 0.18 nmol/µl, 0.15 nmol/µl, 0.12 nmol/µl, and 0.06 nmol/µl. These volumes are summarized in the following table:

Standard Dilution (nmol/µl)	**0.3**	**0.24**	**0.21**	**0.18**	**0.15**	**0.12**	**0.06**
0.3 nmol/µl Standard (µl)	200	160	140	120	100	80	40
Distilled water (µl)	0	40	60	80	100	120	160

For the blank, or 0 nmol/µl standard, use pure Distilled water. The volume of each standard will be 200 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.

B. Assay Procedure

Cell Pre-Treatment

1. Mark the positions of the samples and controls on a blank 96-well microplate. *Each sample well requires a corresponding control well. When measuring the fluorescence of the contents of these wells using the black (fluorescence) microplate provided, it is recommended to test the standards in duplicate. This will leave 80 wells to test 40 samples in duplicate (40 samples + 40 corresponding controls).*
2. Seed the cells in the 96-well microplate, to a density of ~2000 cells per well. At this stage, the cells can be treated according to the needs of the experiment. *Do not seed the cells in the black fluorescence microplate.*

Glucose Uptake Assay

3. Starve the cells in the wells in serum-free cell medium overnight.
4. Discard the serum-free cell medium. Wash the cells twice using 200 µl Buffer Solution with 2% BSA.
5. Add 100 µl of Buffer Solution with 2% BSA to all wells.
6. Add 10 µl Substrate to the sample wells.
7. Add 10 µl Buffer Solution to the control wells.
8. Incubate at 37°C for 30 minutes.
9. Wash the cells 3 times using 100 µl Buffer Solution.
10. Add 50 µl Acidic Reagent to all wells. Leave the plate to stand at room temperature for 10 minutes.
11. Add 50 µl Alkaline Reagent to all wells.

Fluorescence Readings

12. Mark the positions of the standards, samples, and controls on the black fluorescence microplate. *Each sample well requires a corresponding control well. It is recommended to test standards in duplicate.*
13. Add 30 µl of each Standard Dilution to the corresponding standard wells on the black (fluorescence) microplate.
14. Take 30 µl of liquid from each sample well on the 96-well microplate, and transfer to the corresponding sample well on the black (fluorescence) microplate.
15. Take 30 µl of liquid from each control well on the 96-well microplate, and transfer to the corresponding control well on the black (fluorescence) microplate.
16. Add 170 µl of Enzyme Reagent Working Solution to all wells on the black (fluorescence) microplate.
17. Incubate at 37°C for 30 minutes.
18. Measure the fluorescence of each well with a fluorescence plate reader at excitation wavelength 530 nm (emission wavelength 590 nm).

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of glucose taken up in each sample well can be derived with the following formula:

1. Serum and Saliva samples:

$$\text{Glucose Uptake (nmol/µl)} = \frac{F_{Sample} - F_{Control} - b}{a}$$

where:

F_{Sample}	Fluorescence intensity of sample
$F_{Control}$	Fluorescence intensity of control
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)